Reduced Lymphatic Drainage from Hamster Cheek Pouch[1] (35129)

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(Introduced by Sheldon C. Sommers)
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The hamster cheek pouch is regarded as an "immunologically privileged" site, comparable to the brain and anterior chamber of the eye, because of its capacity for prolonged maintenance of foreign tissue grafts. It has been suggested that this property is due to its "alymphatic nature" (1) or "shortage of lymph vessels" (2), since attempts using the classical methods of carbon and vital dyes have failed to demonstrate lymphatic channels in the hamster cheek pouch (1–3). Lymphatic vessels, however, have been demonstrated in the hamster cheek pouch connective tissue by histological means (4), thus contesting earlier work on this subject. We here report that lymphatic drainage from the cheek pouch to its regional, ipsilateral, submental lymph node does indeed occur, but in a delayed fashion.

Methods and Results. Lymphatic drainage from the cheek pouch was investigated by injecting India ink (Pellikan) into its connective tissue and observing egression of dye to the regional cervical ipsilateral or contralateral lymph nodes. Upon injecting 0.5 ml of a 1:4 water dilution of India ink into the everted left cheek pouch wall of 4 anesthetized golden hamsters (*Mesocricetus auratus*) of both sexes (70–80 g) and then replacing the cheek pouch to its original position, a brownish-black coloration of the ipsilateral submental lymph nodes was seen within 6 hr. Extensive dissection for other blackened nodes revealed that the dye had spread by diffusion to other areas of the oral cavity and neck, resulting in the involvement of cervical and axillary nodes.

In an attempt to exclude the experimental error of dye leaking out of the cheek pouch proper to adjacent tissues, which apparently was not controlled in the work of Miotti (5) and of Shepro *et al.* (1), we constructed an isolation chamber for the cheek pouch. The left neck contents and cheek pouch were exposed by a longitudinal cut made from the mandible to the 2–3 rib. The left cheek pouch was everted, carefully injected by means of a 30-gauge needle with 0.1 ml of diluted ink, and then replaced with care. Thereafter, a plastic ring having a transparent cover on one side was sewn to the skin of the neck opposite the site of injection, exposing the cheek pouch and ventral neck contents (Fig. 1). An elongated piece of foam rubber was then inserted into the cheek pouch between its double membrane to expand the pouch, absorb excess dye within, and make the injection site more prominent, but without distorting its microcirculation. The dorsal aspect of the cheek pouch was separated from the deep neck tissue by insertion of a thin plastic membrane behind the pouch. This transparent, moistened chamber permitted continuous observation of the isolated cheek pouch *in situ* and its regional cervical lymph nodes. The chamber did not hinder the animal in its movements, and was not damaged during the observation period if particularly docile animals were selected and housed in individual cages. Two additional groups, of 4 hamsters each, received similar injections of India ink to the left side of their upper lips or to their tongues, in order to compare egression of dye from the cheek pouch to that from adjacent sites. The neck contents of these animals were thereafter sur-

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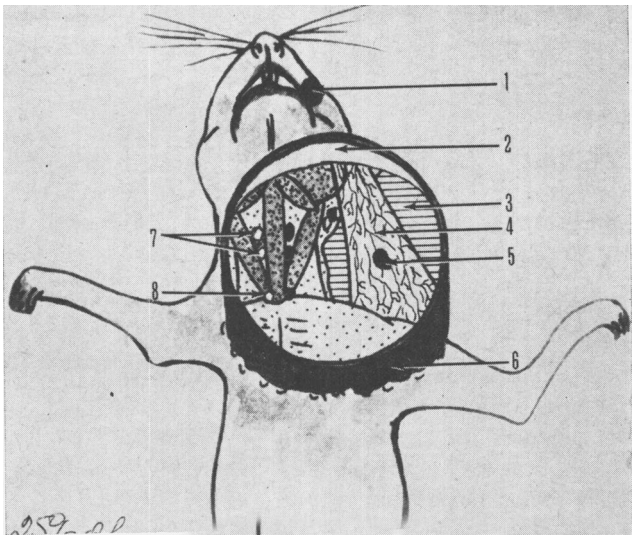

FIG. 1. Cheek pouch isolation chamber showing deposit of India ink and important regional neck lymph nodes: (1) injection site of lip; (2) wall of plastic ring; (3) plastic sheet behind cheek pouch; (4) richly vascularized cheek pouch; (5) ink spot in cheek pouch; (6) chamber sewn to hamster skin; (7) submental lymph nodes; (8) deep cervical, caudal, lymph node.

gically exposed at regular intervals and examined for blackened nodes.

A brownish-black coloration of only the ipsilateral submental lymph nodes was first observed 20 hr after injection of India ink to the isolated cheek pouch (Fig. 1). Distinct lymphatic channels in the pouch, however, could not be seen. The deposit of ink in the cheek pouch wall spread about 3–4 mm in all directions and seemed to be somewhat hindered in its further expansion, even during a period of 2 days. The hamsters injected with ink into their lips or tongues showed intensely blackened submental and cervical lymph nodes on the ipsilateral side within 2 hr.

Discussion. The studies of Billingham and Silvers (6–8) have divulged that the immunologically unique capacity of the hamster cheek pouch for prolonged acceptance of allogeneic and xenogeneic grafts is in some way related to a capacity of its loose areolar connective tissue component to impede the evocation of an immunological response. Once prior sensitization of the hamster is accomplished by other routes, however, the usual process of graft rejection follows even for cheek pouch skin allografts. Thus, Billingham and Silvers point to the afferent arc of the immunological reflex (sensitization process) as the deficiency reflected by the cheek pouch's immunological peculiarity. Moreover, cheek pouch grafts have an added advantage over the host's defenses because of this site's rich and pliable vascular network (9, 10), permitting adequate graft nourishment.

In his paper on the lymphatic system of the golden hamster, Miotti (5) states that the submental nodes drain this area, but without explicitly saying that he indeed injected India ink into the cheek pouch, a point of criticism already raised elsewhere (1). The only direct proof that the cheek pouch does contain lymph vessels is the histological evidence of Lindenmann and Strauli (4), which, it should be added, contradicts the statement by Shepro *et al.* (1) that lymphatics "cannot be seen in routine histological preparations." Whereas we have observed vessels in the cheek pouch resembling those depicted by Lindenmann and Strauli, we cannot unequivocally state, on pure morphological grounds, that these are truly of a lymphatic nature.

The findings reported here, however, lend support to the thesis that there are lymphatics in the hamster cheek pouch and militate against the view (1) that no direct route

exists between the cheek pouch and its draining lymph nodes. We are unable to explain, however, why distinct lymph channels cannot be demonstrated in the cheek pouch proper by means of India ink or vital stains.

Compared to the time necessary for egression of dye from other areas of the hamster head, it appears that there is a delayed lymphatic drainage from the cheek pouch, by a factor of 10. This time factor, as well as a quantitative reduction in drainage, was further confirmed by means of histochemically staining the ipsilateral submental lymph nodes of hamsters injected with iron into their cheek pouch (11). An impeded spread of dye within the cheek pouch connective tissue was also witnessed here, which might be an important factor in the delayed drainage of antigenic material from this site, in accordance with Billingham and Silvers' connective tissue "barrier" hypothesis (6–8). On the other hand, it remains to be clarified whether the lymph vessels seen in histological sections are fewer in number or of the same functional capacity as those present elsewhere in the hamster. Indeed, Shepro *et al.* (12) did report that bacteria escaped from the cheek pouch at a slower rate than bacteriophage, suggesting that antigen drainage from this site is also related to particle size.

Summary. Using a newly constructed, transparent isolation chamber preventing diffusion of India ink to adjacent tissues of the hamster mouth and neck, egression of dye to the regional, ipsilateral, submental lymph nodes takes place, but in a reduced fashion. It is suggested that the so-called "immunologically privileged" character of the hamster cheek pouch is related to a delayed and reduced lymphatic drainage from this site.

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